Infection

From Worms to Nucleotides

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Grant-giving bodies often use the word *timely*, sometimes coupled with the word *promise*, to describe the principles used in allocating money. Research cannot be undertaken on certain themes until technical restraints have been removed, namely until it becomes possible to attain certain levels of cold, heat, magnification or pressure. Even a superficial survey of some aspects of the history of biology suggests that, apart from such physical constraints, *timeliness* is less a statement about a research project, than about the vision and receptiveness of the grant-giving body to which the project is submitted. A symposium with the title 'Of timeliness and promise – sterile and fruitful approaches in the development of biochemistry' (December 1978), gave an opportunity for assembling a few examples, in the general domain of infection, of misjudged timeliness. This is a slightly expanded version of the paper given at the symposium.

'I would use (these weapons) and avenge Patroclus, were it not that flies might settle on his wounds during my absence, breed maggots, and cause decay.' That is Robert Graves' translation of a conversation at the beginning of Book 19 of the *Iliad*. Thetis, who had given her son, Achilles, the weapons, then reassures him that she will embalm the corpse with 'red nectar and ambrosia' so that he need not sit by it to keep off flies, but can take part in the fighting outside Troy. Homer, or the syndicate that goes by that name, made a perfectly clear statement that has remained in the *Iliad* for nearly 3000 years because it accords with common experience in farm, kitchen and tomb.

We may wonder therefore what was new in the experiments published by Redi in 1688.[1] He found that uncovered pieces of meat, from a score of different animal species, produced maggots of the same type, but he found none on covered meat. He claimed no originality; butchers and hunters usually covered meat at that time to keep off flies. In spite of the near-universality of classical education, the point had escaped the attention of scientists and philosophers. They concocted the idea of spontaneous generation. Having bedevilled biology for a millennium, it still caused trouble for two centuries after Redi.

Many points meriting discussion arise from that story. Why have maggots, or worms to use a general term common even after Linnaeus, played such an important role in discussions about the causation of disease, the preservation of food, and the origins of life? What is the nature of discovery? Were Homer and Redi discoverers, because they traced a rational connection between protection from flies and the elimination of maggots, whereas butchers and hunters may merely have been following a ritual or superstition? People who are not experimentally minded 'know' many things – only a fraction of them true. Separating fact from fiction is as truly discovery as noticing something that no one had suspected before.

As Josh Billings remarked 'It ain't what a man dont know that makes a fool of him, it's what he do know that ain't so.' Finally, why do perfectly definite observations, and not just suggestions and interpretations that are later found to have merit, so often lie neglected for decades or centuries? The many examples that crop up in the history of our knowledge of various aspects of even a single subject, such as infection, make it legitimate to wonder whether the concept of 'timeliness and promise', enshrined in many statements from Research Councils, is not bogus. Within broad limits, any project which is within the compass of the physical equipment of the time, is timely if there is an enthusiast eager to pursue it.

WORMS

Until about the beginning of the 19th century, movement was the usual criterion separating living from non-living structures. This attitude is shown by the word *viviparous*, the phrase 'the quick and the dead' and Coleridge's comment that scientists 'could not hear the life of metals asserted with a more contemptuous surprise than they themselves incur from the vulgar, when they speak of Life in mould or mucor'.[2] Worms are indubitably alive because they wriggle; and they are obviously connected with mortality.

In the confused history of the development of ideas about infection – the colonization of an organism by something that multiplies within it – worms have an important place. Fracastor[3] seems to have been the

CCC–0308–0188/80/0005–0312$04.00/1

first to emphasize the significance of multiplication as a means of distinguishing infection from poisoning. Aulus Cornelius Celsus, Rome's greatest medical writer (about 50 AD) may have had a glimmer of this idea; when writing of rabies he used the word *virus*, but for snake poison he used *venenum*. Fracastor introduced the word *fomes* for the material that he suggested was the transmitter of disease from person to person; he recognized that each infection had a specific agent and that this multiplied in the host, but he did not explicitly say that the agent was alive. That generalization had to wait for the 17th century. Then, magnifying lenses, and the urge to use them, became timely.

Reliance on unaided vision is now amazing to us, when so many even before old age makes it hard to read small print, have a lens among our pocket paraphernalia and use it when anything interesting is noticed. Shakespeare probably did not use a lens. One version of his comment on the scabies mite runs:

> . . . a little worme,
> Pickt from the lasie finger of a maide,
> (Romeo and Juliet, Act I, sc. iv).

But Redi used one to study mite anatomy in detail. Extensive use was advocated by Glanville,[4] who wrote in his *Plus ultra* (1668) 'There is an inexhaustible variety of Treasure which Providence hath lodged in Things, that to the World's end will afford fresh Discoveries' and went on to mention the '. . . little Threds and Springs . . . ' in an organism specifically.

EARLY MICROSCOPISTS

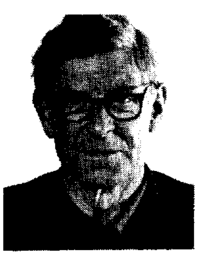
Athanasius Kircher[5] was already an enthusiastic microscopist. He saw worms everywhere and was largely responsible for what Singer called the 'vermicular obsession' of the 17th century. Some went so far as to attribute all disease, indeed even 'natural' death in man and animals, to worms. Bearing in mind how much of early medicine was developed in warm countries where maggots develop quickly, this was not wholly unreasonable. Nevertheless, Avicenna thought that worms could be beneficial – an interesting forerunner of the occasional use during the present century of fly maggots for removing dead tissue from a wound.

Whether or not worms were responsible for disease, Redi had shown that maggots were not spontaneously generated; others doubted the spontaneous generation of worms elsewhere. Thus Boyle, in *The sceptical chymist* (1661), makes Carneades say '. . . common water . . . will putrifie and stink, and then perhaps too produce moss and little worms, or other insects, according to the nature of the seeds that were lurking in it'. Nevertheless, the idea remained untimely for a further 200 years. Because of a rich accretion of philosophical, political and theological associations, it was discussed with a level of acrimony unusual in scientific debate. Tapeworms dominated an entertaining phase of the wrangle. Ecclesiastical dogma seems to have been clear. No new species were created after the Sixth Day; the spontaneous generation of anything as elaborate as a tapeworm was therefore contrary to Holy Writ; Adam and Eve were perfect before the Fall and therefore could not have had worms or even a tendency to develop them for later transmission. The problem seemed insoluble until the essential intermediate hosts of these parasites were discovered in the 1840s. Interest in the place of worms as the cause or consequence of disease spread into literary circles; Addison, Defoe and Swift comment on worms seriously or humorously.[6]

At about the time when belief in worms and insects generally as causes of disease was beginning to wane, the idea started that they could be vectors. Malaria was associated with insects by Lancisi,[7] and diseases that were probably bartonellosis and leishmaniasis were associated with sand flies by Bueno.[8] These associations were more specific than the vague condemnation of insects by such Roman writers as Varro and Columella – the latter listed biting insects, along with visitors, among the hazards of living beside the main road! These 18th century correlations were about as good as those that started Reed and Ross on the control of yellow fever and malaria. The long interval is interesting. Perhaps it was needed for insects to recover timeliness after the demise of the 'vermicular obsession', perhaps delay was caused by the intellectual difficulty of separating the roles of vector and infective agent.

GERMS

As improvements in microscopy enabled more of the internal structure of the supposedly simple organisms



N. W. PIRIE, FRS, worked in Sir F. G. Hopkins' laboratory in Cambridge, UK, for 11 years on the isolation, metabolism and behaviour of glutathione, methionine and other S compounds; on the antigens of *Brucella*; and on the isolation of plant viruses. He moved to Rothamsted in 1940 primarily to facilitate virus research, but the move enabled work on the extraction of edible protein from leaves to be conducted on a larger scale than was possible in Cambridge. From 1947 to 1973 he was head of the Biochemistry Department at Rothamsted. On retirement, work on plant viruses ended. Work on leaf protein continues – especially on the design of simple and robust equipment that could be used for making it by relatively unskilled people.
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to be seen, it became increasingly improbable that such elaborate structures could simply assemble themselves. By the beginning of the 18th century, most biologists had come to agree with Homer and Redi. But fermentation remained a problem. Leeuwenhoek asserted that the 'beasties' he studied arose from other organisms only; he saw yeast cells, but did not assign them a role in fermentation. Boerhaave[9] 'discovered' that fermentation started more quickly if material from an old brew was added to the wort – brewers had, of course, known this for millennia. Yeast cells were not admitted to the category of living organisms until Cagniard-Latour[10] described their reproduction by budding. Chemists, like Liebig and Wöhler, rejected the idea with contumely and ridicule, but it led to the well-known series of experiments, notably by Pasteur, on the precautions needed to keep fermentable fluids from fermenting. The fact that air carried the particles needed for the initiation of fermentations and putrefactions, started Pasteur on the search for identifiable agents, rather than vague concepts such as contagion and miasma, as causes of disease. In this he was following Bassi who, 30 years earlier,[11] had transmitted *muscardine* to silk worms with a needle, and elaborated from his experiments a reasonably coherent germ theory of disease.

To a large extent many of us still depend on the sewage systems and clean water supplies installed in the middle of the 19th century. Tentative moves toward slum clearance were also made then. Those responsible for these improvements, Simon, Snow,[12] Nightingale, Virchow and others attached little importance to micro-organisms though they were aware of their existence. There is a tendency now to attach too much importance to the purely microbiological consequences of the reforms. The reformers had a more Hippocratic approach and were concerned with the general phenomena of dirt, air, food and the standard of living. As Feuerbach – usually remembered only because Karl Marx attacked him – put it 'Do you want to improve people? Then, instead of preaching against sin, give them better food'.

As so often in the history of science, trouble was caused by false antitheses and the difficulty of distinguishing a necessary, from a necessary and sufficient, condition. Instead of seeing that personal constitution, the nature of the environment and the presence of an infective agent all played a part in the final appearance of an infectious disease, many people argued and still argue, for one factor to the exclusion of the others. As recently as 1847, Semmelweiss was ostracized for telling doctors to wash more often, and washing was counted as one more bit of heterodoxy in Paracelsus' (1493–1541) turbulent career; it has now become timely.

When Lister and Tyndall[13] published articles in the *British Medical Journal* on the suppuration of wounds and the role of airborne germs in human disease, they felt it wise to point out that there was no conflict between their preoccupation with the immediate environment of the patient, and the sanitary reformer's interest in the environment as a whole. They stressed, as Fracastor had done, the importance of multiplication. Lister tried to avoid conflict by saying that it did not really matter whether the 'septic particles' were organisms. However, he thought they were (see Figs. 1 and 2). In the course of a long account of operations done aseptically, he said 'That they are self-propagating, like living beings, and their energy is extinguished by precisely the same agencies as extinguish vitality . . . is certain, and is of the utmost practical importance'.

Tyndall was more doctrinaire and wrote 'Now it is in the highest degree important to know whether the parasites in question are spontaneously developed, or are wafted from without to those afflicted with the disease. The means of prevention, if not of cure, would be widely different in (the) two cases'. The medical profession remained, for a time, unimpressed. At an International Sanitary Conference in 1885, Koch's recent discovery of the cholera vibrio was not mentioned. However, in spite of the scepticism of Pettenkofer and Metchnikoff, who ostentatiously drank cultures supposedly containing the vibrio, the infectivity of cholera was accepted in 1892.

There are few better examples of compartmentalized thinking than the almost complete absence from all this discussion of any mention of what was going on in the kitchen. Sausages were preserved with spices less exotic than Thetis' 'red nectar and ambrosia', but sausages needed only temporary preservation! The use of thin pastry casings is more relevant to the general theme of infection. Every cook knew that an unopened pie or Cornish pasty could be kept for longer than an open stew. Cooks did not, obviously, realize that this was because the dry casing excluded micro-organisms from the moist contents; it is odd that scientists did not think about the matter.

In 1732, C. Carter published 'The Compleat City and Country Cook' (see Fig. 3). This memorably alliterative work describes in some detail what is in essence the modern method of bottling fruit. An anonymous book of receipts had described the bottling of gooseberries in 1680. As a result, 'Gooseberry bottles' were made until late in the 18th century in Europe when restrictions on certain raw materials made it impossible to produce in Britain glass of suitable quality. At the end of the 18th century the experiments of Spallanzani provided an explanation for the efficacy of these techniques. In 1808, Saddington received a five guinea prize from the Society of Arts for exhibiting twelve fruits preserved for two to three years in wide-mouthed wine bottles. In the light of all that, it is hard to see what was so novel about Appert's book[14], which is usually cited as having started the food preservation industry. It is even harder to see why the demise of spontaneous generation did not become timely until after the later work of Schwann, Pasteur and Tyndall. It can be

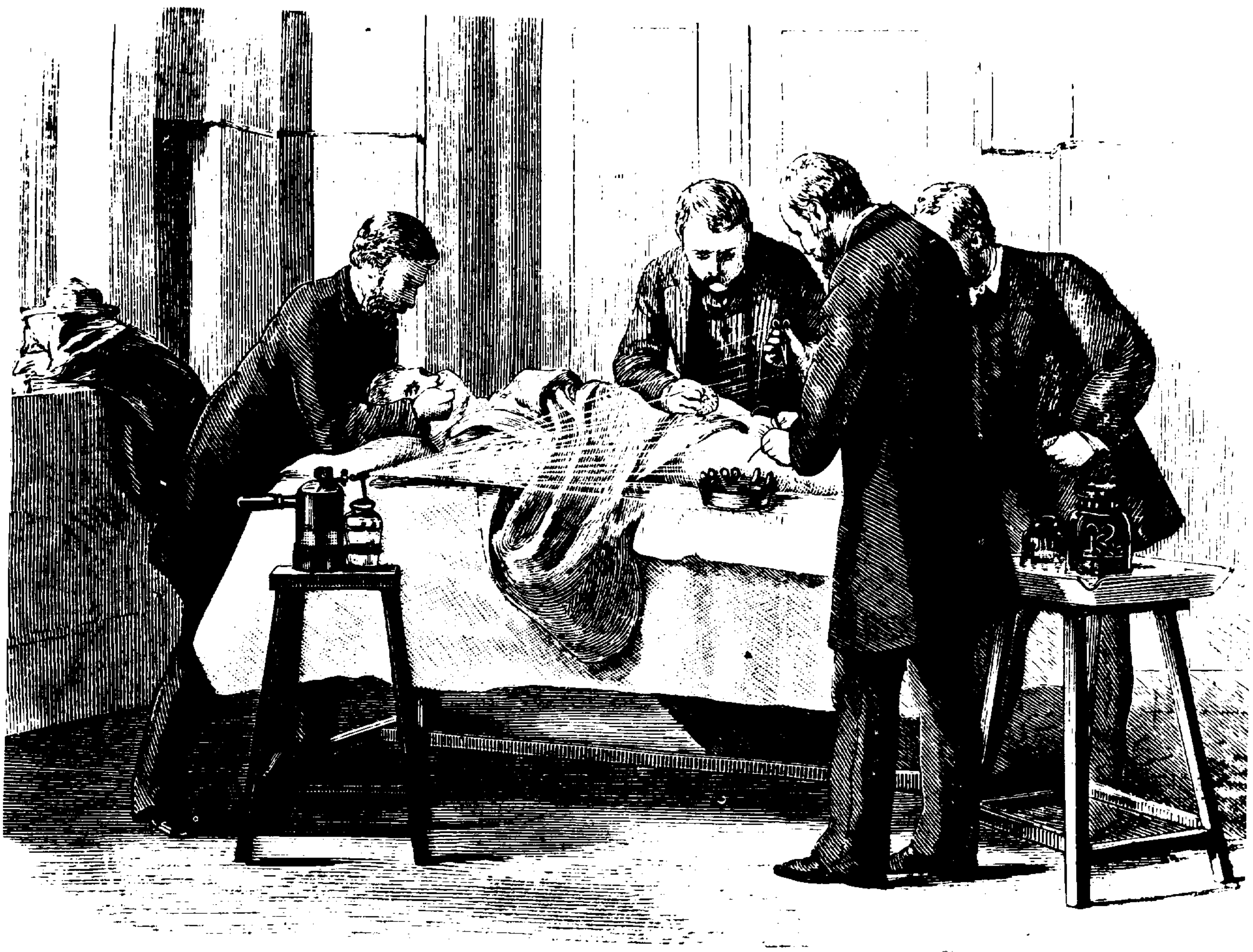

Figure 1. The Lister carbolic acid spray in use. From W. Watson Cheyne, *Antiseptic Surgery*, London (1882). Courtesy of the Wellcome Trustees.

argued that Pasteur and the others did little more than teach scientists what housewives already knew.

VIRUSES

Pasteur, like his contemporaries, used the word virus loosely; when in 1889 his microscope revealed nothing in a fluid transmitting rabies, he postulated an organism similar to those he was familiar with – but smaller. Filters that were supposed to retain bacteria were already used at that time so Loeffler and Frosch,[15] who were trying to make a vaccine to protect cattle against foot and mouth disease, were surprised by the infectivity of a filtrate. By simple arithmetic they demonstrated that they were dealing with an agent that multiplied, and not with a toxin. In the same year, Beijerinck came to the same conclusion about tobacco mosaic virus. Although he was a lifelong friend of the physical chemist van't Hoff, he confused matters by calling the agent 'fluid' and thus set pathologists off on a metaphysical wild goose chase about 'soluble substances' and 'particles' from which some have not yet returned. By accident these agents, and others found soon afterwards, were called ultramicroscopic or filterable viruses rather than bacteria: the designation, without the prefix, has stuck.

Although these viruses multiplied in susceptible hosts, they did not multiply in cell-free culture media. Beijerinck stressed this correlation, as did Landsteiner[16] who dismissed, on the grounds that the medium contained nucleated erythrocytes, the supposed cell-free cultivation of fowl plague virus. The generalization that viruses, because of their absolute dependence on a host, cannot usefully be classed as organisms, did not become timely for a further 40 years. The implications of the generalization have not yet been fully appreciated: viruses are still sometimes introduced into discussions on the origins of life. The dependence of viruses on a host was an untimely idea; equally untimely observations were being made at that time. Copeman and Centanni cultivated viruses in embryonated eggs, and Landsteiner agglutinated erythrocytes with fowl plague, a form of influenza, virus. Both techniques were commercialized a generation later (see Fig. 4).

Bacteria, like plants and animals, vary in composition according to their age and state of nutrition. It is reasonable to expect that the scope for variability will

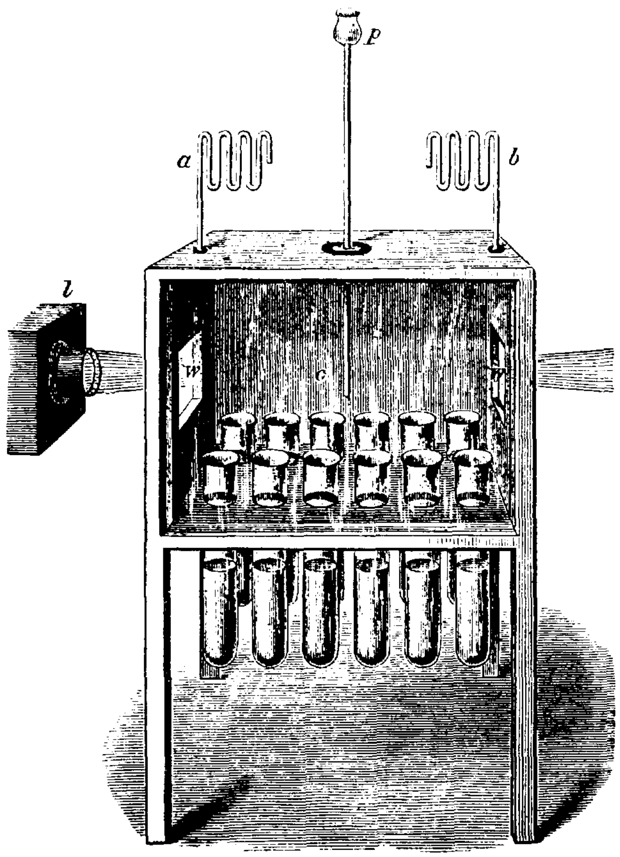

Figure 2. Tyndall's box in which he studied the relationship between contamination of the bacterial culture medium in the glass tubes and the presences of particles in the air. The box contained a glass front window (C) as well as two smaller windows (W) through which a strong light could be projected thus making the particles visible. (l) is the lens mount, (a) and (b) glass tubes to prevent entrance into the box of unwanted particles and (p) funnel to fill the tubes. Reproduced from *Essays on the Floating Matter of Air* London (1883) courtesy of Ann Ronan Picture Library; this illustration was first published by Tyndall in *Philos. Trans. R. Soc. London* **27**, 166 Part 1 (1876).

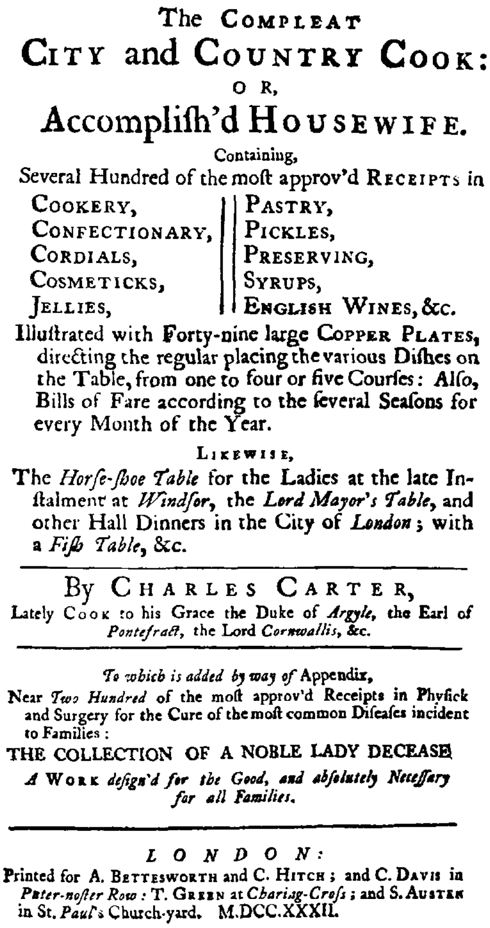

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Figure 3. Title page of Carter's famous book *The Compleat City and Country Cook,* London (1732). Courtesy of The British Library.

be smaller, the smaller the infective agent. When it is an obligate parasite, in a sense a metabolic product of the host, it is still more likely to have constant composition. These expectations, coupled with the tendency of viruses to be more resistant to chemical insuit than bacteria, made it reasonable to try to purify viruses in the chemical sense. Fowl plague virus was called a globulin by Mrowka,[17] and fowl pox virus a nucleoprotein by Sanfelice.[18] The evidence that Schlesinger[19] published for the nucleoprotein nature of a bacteriophage was much more convincing and prompted Bawden and Pirie[20] to make the cautious comment on Potato virus X '. . . protein is an essential part of virus 'X', but there is no evidence that other equally important substances may not also be present'.

Tobacco mosaic virus was called a globulin by Stanley;[21] we found[22] that it was a nucleoprotein and went on to show that a dozen other plant viruses, or virus strains, were also nucleoproteins. The presence of nucleic acid was first disputed; it was then neglected because of the widespread illusion that nucleic acids were tetranucleotides and so too small to carry much specificity. Our suggestion[23] that the nucleic acid was just as likely as the protein to be important, was interpreted as our nucleic acid obsession in spite of the evidence, marshalled by Avery and his colleagues[24] that the pneumococcal transforming factor was nucleic acid. Nucleic acids began to be considered timely when Hershey and Chase[25] found that more nucleic acid than protein went into a bacterium when it was infected by a bacteriophage. The job was completed when Fraenkel-Conrat and Schramm infected plants with the nucleic acid separated from tobacco mosaic virus. The inoculum had to be stronger than usual, but this relegated protein in viruses to a protective rather than a primary function and gave nucleic acids their present dominant role in biochemistry.

SOME REASONS FOR DELAY

Throughout this account of the growth of knowledge about processes of infection, I have commented on

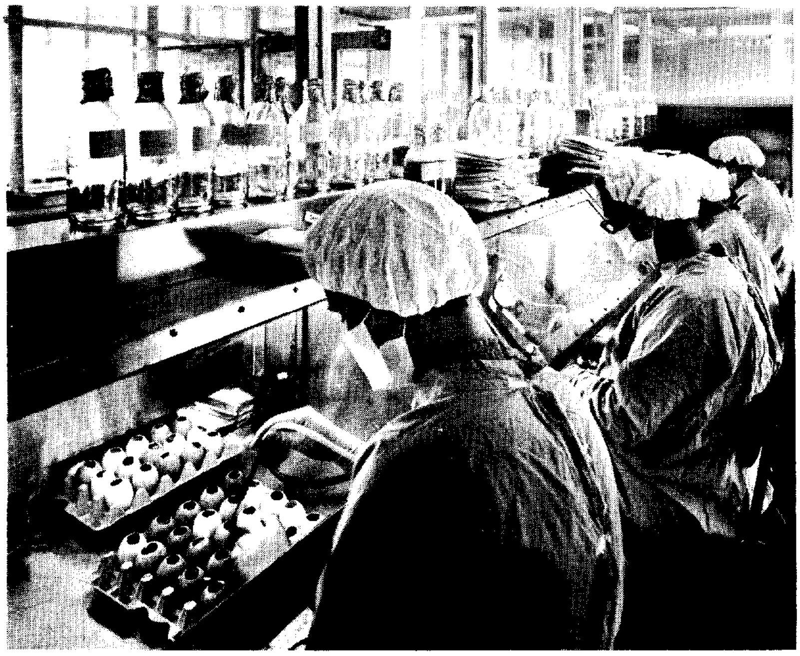

Figure 4. The cultivation of viruses in embryonated eggs. The eggs are inoculated with 0.2 ml of diluted virus suspension into the allantoic cavity, an automatic syringe being used (Left). After 2–3 days incubation, the eggs are transferred to a cold room to chill the embryos. After a suitable period of time, the eggs are swabbed with a meth/iodine solution and a circular cut is made in the shell over the air sac with a trephaning machine (Right). Courtesy of Duncan Flockhart and Company, London E2.

the fact that several lines of research would have been timely decades or centuries before they were pursued. This review may therefore end with some comments on the general topic of delay. Acceptance of ideas and observations may, understandably, be delayed until developments in related sciences have supplied a congenial intellectual environment. To a casual observer it seems that Mendel's observations on inheritance had to await the rise of biometry, Waterston's approach to the gas laws had to await unequivocal evidence for atomicity, and Wegener's suggestions about geodynamics had to await better bathymetric and magnetic surveys. The list could easily be extended.

Popular histories of science contain so many stories of vigorous opposition to ideas which later gained general acceptance, that it is tempting to think that sound novel ideas are sure to be opposed. The thought is sometimes inverted: opposition or neglect has been regarded as evidence for the soundness of an idea. But most novel ideas are wrong. The situation is different with an observation. It may be misinterpreted, but it cannot be wholly wrong. Thus, to take an example connected with the theme of infection, in some communities a priest blesses a new building in which cheese will be made. Part of the ritual involves rubbing it with old cheese. Presumably the ritual works: which is the effective part of it is a matter of opinion and for later experiment. When a technique is observed to work, it is hard to understand why the attempt to find out the reason is so often delayed.

Louis Racine (*fils*) remarked '. . . in order better to humiliate those who cultivate the sciences, God has permitted the finest discoveries to be made by chance, and by those least apt to make them. The sea compass was not invented by a mariner, nor the microscope by a physicist, nor printing by a writer, nor gunpowder by a soldier'.[26] We can add that Priestley was a non-conformist minister, Boussingault a mining engineer, Pasteur a crystallographer, and the first observations on current electricity were made by biologists. This sort of thing annoys specialists. They think, naturally, that advances in their subject should be made by them. The argument goes back a long way in both time and epistemology. Posidonius, who was a philosopher, said that innovations were made by philosophers: Seneca (90th Epistle) said they were made by workmen. In *The fable of the Bees*, Bernard de Mandeville discussed the issue and agreed with Seneca, so did Adam Smith in *The Wealth of Nations*.

When outsiders make suggestions or observations, opposition or neglect are understandable. Disregard for observations made by people with orthodox qualifications, and on subjects later given great importance, is harder to understand. The 'Non-conservation of Parity' is an excellent example with biological implications. The Nobel Prize for physics was awarded to Lee and Yang within a year of their observation of chirality in some atomic processes. Research on the subject was then undertaken with such enthusiasm, or mania, that another physicist had to withdraw five papers because his dream world had intruded on his experiments! Franklin,[27] in a thoughtful discussion of the whole matter, points out that similar observations had been reported, and neglected, 29 years earlier.

Fortuitously, newspaper accounts of Lee and Yang's observation coincided with a New York Academy of Sciences symposium on 'Modern ideas on spontaneous generation' held at the end of 1956. Pasteur regarded the preferential use of only one of the isomers of a chiral molecule as a peculiarly biological phenomenon. During the 1930s it became clear that, although individual members of a group of molecules such as the amino acids rotated the plane of polarization of light in either direction, all members of the group had the same configuration in 3-dimensional space. The origin and significance of chiral preference was therefore regularly discussed in papers on biopoesis. The possibility that it was the consequence of some fundamental atomic asymmetry was therefore an important subject of conversation of those attending the symposium. The sudden excitement about parity in physics laboratories can hardly be explained by this biological interest because that did not emerge in print until 1960.[28] All that Franklin feels able to conclude is that '... not only the physical and logical content of an experiment determines whether it is crucial or not.'

From one point of view, the present-day influence of think-tanks and the increasing control of research by civil servants could be beneficial. It is often easier to get a non-specialist than a specialist to understand the essence of a new idea, or the significance of an old observation – fewer layers of habit and conventional wisdom have to be peeled away. On the other hand, the non-specialist usually submits the matter to a specialist committee and, unless very obstinate, accepts condemnation by the committee. The basic fallacy underlying the British customer/contractor principle of financing research is the assumption that the customers know enough about what it might be possible for them to get, to know what to ask for. That is the sort of knowledge that people who actually handle material and equipment are most likely to have. The point was well put by Mees, who directed research for Eastman Kodak.

'The best person to decide what research shall be done is the man who is doing the research. The next best is the head of a department. After that you leave the field of best persons and meet increasingly worse groups. The first of these is the research director, who is probably wrong more than half the time. Then comes a committee which is wrong most of the time. Finally there is a committee of company vice-presidents, which is wrong all the time.'[29]

That may be an extreme point of view. Nevertheless, it recognizes the fact that developments are most likely to come from people with practical experience, especially if that experience extends to what is happening in factory, farm and kitchen.

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The manuscript was received 6 June 1980

Fumigation of the special artist of the *Illustrated London News* when covering the Hamburg cholera epidemic in 1892. From *Illustrated London News*, 17 September 1892. (Courtesy Ann Ronan Picture Library.)